Cold Tolerance and the Regulation of Cardiac Performance and Hemolymph Distribution in *Maja squinado* (Crustacea: Decapoda)

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ABSTRACT

Elevated Mg^{2+} levels in the hemolymph ($[Mg^{2+}]_{HL}$) of brachyuran crabs have recently been demonstrated to limit cold tolerance by reducing motor and circulatory activity. Therefore, the limiting function of elevated $[Mg^{2+}]_{HL}$ on circulatory performance and arterial hemolymph flow was investigated by the pulsed-Doppler technique in the spider crab *Maja squinado* during progressive cooling from 12° to 0°C. $[Mg^{2+}]_{HL}$ were reduced from control levels of 39.9 mmol L^{-1} to levels of 6.1 mmol L^{-1} by incubation in magnesium reduced seawater. At 12°C cardiac output was 13.9 ± 2.4 mL kg^{-1} min^{-1} and stroke volume 0.2 ± 0.04 mL kg^{-1} min^{-1} in control animals. In $[Mg^{2+}]_{HL}$-reduced animals cardiac output increased to 43.6 ± 5.0 mL kg^{-1} min^{-1} and stroke volume rose to 0.6 ± 0.1 mL kg^{-1} min^{-1}. Temperature reduction in control animals revealed a break point at 8°C linked to a major redirection of hemolymph flow from lateral to sternal and hepatic arteries. Cardiac output and heart rate dropped sharply during cooling until transiently constant values were reached. Further heart rate reduction occurred below 4.5°C. Such a plateau was not detected in $[Mg^{2+}]_{HL}$-reduced animals where the break point decreased to 6°C, also indicated by a sharp drop in heart rate and cardiac output and the redirection of hemolymph flow. It is concluded that progressive cooling brings the animals from a temperature range of optimum cardiac performance into a deleterious range when aerobic scope for activity falls before critical temperatures are reached. Reduction of $[Mg^{2+}]_{HL}$ shifts this transition to lower temperatures. These findings support a limiting role for $[Mg^{2+}]_{HL}$ in thermal tolerance.

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Introduction

In various marine invertebrate species cold tolerance is limited by a low critical temperature characterized by the transition from aerobic to anaerobic metabolism (Zielinski and Pörtner 1996; Sommer et al. 1997; Pörtner et al. 1998). In the spider crab *Maja squinado* (Herbst), on-line hemolymph Po_2 measurements revealed that oxygen tension remained constant during cooling until a transition from optimum to a deleterious range occurred. This progressively deleterious temperature range ("pejus range," "pejus" meaning "turning worse") was characterized by a fall in Po_2 and aerobic scope for activity (Frederich and Pörtner 2000). Finally, oxygen supply to tissues by ventilation and circulation was suggested to become limiting, thereby eliciting the onset of anaerobiosis at the critical temperature.

This scenario implies that not only oxygen uptake at the gills but also maintenance of hemolymph flow is critical for thermal tolerance. In this context, cardiac output is the crucial parameter of cardiac activity. In earlier studies heart rate was selected as an indicator for cardiac performance (e.g., Florey and Kriebel 1974; Cumberlidge and Uglow 1977; Hamilton and Houlihan 1992); however, heart rate, cardiac output, and stroke volume may not be strictly correlated (Wilkens 1987; McMahon and Burnett 1990; McGaw et al. 1994, 1995; McMahon 1999). Previously, cardiac output was determined by the Fick principle or the thermodilution technique (Burnett et al. 1981; McMahon and Wilkens 1983). Direct on-line monitoring of hemolymph flow through individual blood vessels is possible with minimal handling stress by use of the pulsed-Doppler technique. Therefore, this technique has been established for the determination of cardiac output and stroke volume in brachyuran crabs (Airriess et al. 1994).

Recent work has shown an inverse correlation between activity levels and Mg^{2+} concentration in hemolymph ($[Mg^{2+}]_{HL}$; Frederich et al. 2000*a*). Because Mg^{2+} acts as an anesthetic, especially at low temperatures, it has been hypothesized that the ability to regulate $[Mg^{2+}]_{HL}$ influences cold tolerance (Frederich et al. 2000*a*). Reptant brachyuran crabs possess high magnesium levels in the hemolymph ($[Mg^{2+}]_{HL}$ 30–50 mmol L^{-1}),

whereas caridean shrimps possess low $[Mg^{2+}]_{HL}$ (6–12 mmol L^{-1}). Experimental reduction of $[Mg^{2+}]_{HL}$ to shrimplike values (8 mmol L^{-1}) increased activity and cold tolerance in brachyuran crabs like *Hyas araneus* (Frederich et al. 2000*a*).

This hypothesis together with the assumed key role of circulatory performance in thermal tolerance led us to investigate the dependence of cardiac performance on temperature and $[Mg^{2+}]_{HL}$ in more detail. The temperate species *M. squinado* proved suitable to investigate this question because the limiting effects of low temperature at various $[Mg^{2+}]_{HL}$ could be investigated at a higher temperature resolution than in a cold-adapted species. First, we compared photoplethysmograph signals with Doppler analyses of cardiac output to test whether noninvasive heart rate recordings can be used to reliably determine changes in cardiac performance. Second, we investigated changes in cardiac performance during progressive temperature reduction to extremely low values. Third, we analyzed the limiting function of $[Mg^{2+}]_{HL}$ for cardiac performance. In this way, we tested the hypothesis that Mg^{2+} regulation may be an important factor for setting the lower limits of thermal tolerance.

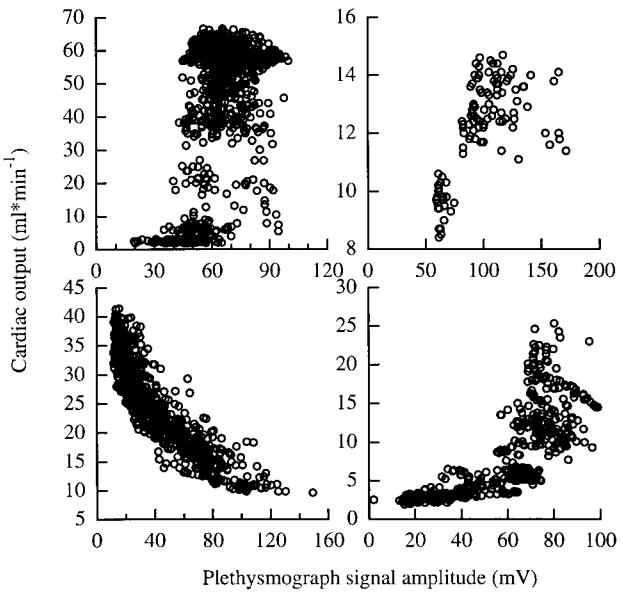

Figure 1. Comparison of photoplethysmograph signal amplitudes and cardiac output measured with the ultrasonic pulsed-Doppler technique in four specimens of *Maja squinado*. The correlation differs completely between animals and allows no reliable assessment of cardiac performance based on signal amplitude.

Material and Methods

Animals

Adult male and female *Maja squinado* with a mean weight of 376 ± 86 g were purchased from local fishermen in Roscoff, France, and held in large tanks with recirculating natural seawater at 12° ± 0.2°C and 33‰ salinity at least 2 wk before the start of the experiments. They were fed twice a week with pieces of cod (*Gadus morhua*) and mussels (*Mytilus edulis*).

Experimental Procedure

Maja squinado is a poor Mg^{2+} regulator ($[Mg^{2+}]_{HL}$ 39.9 ± 4.6 mmol L^{-1}, $[Mg^{2+}]_{seawater}$ 53 mmol L^{-1}), and it is easy to modify $[Mg^{2+}]_{HL}$ by exposure to Mg^{2+}-reduced artificial seawater (Aquarium Systems, Sarrebourg, France; ion composition in mmol L^{-1}: Na^+ 487, K^+ 10, Ca^{2+} 10, Cl^- 490, SO_4^{2-} 27, Mg^{2+} 6, pH 8.0). Hemolymph samples were obtained by inserting a cannula into the articular membrane at the coxa of the last walking leg. $[Mg^{2+}]_{HL}$ was determined photometrically (Merckotest Magnesium, Merck, Germany). After 3 d of exposure $[Mg^{2+}]_{HL}$ was constant at 6.1 ± 0.7 mmol L^{-1}.

Animals equipped with photoplethysmographs and Doppler probes (see below) were kept in natural or Mg^{2+}-reduced seawater in a temperature-controlled darkened 25-L aquarium at 12°C for 15 h before temperature change. Animals were allowed to move freely with chelae covered by pieces of tubing to prevent destruction of the Doppler probes. For an analysis of the effect of progressive cooling temperature was reduced to 0°C over a 12-h time period at 1°C h^{-1}.

Hemolymph Flow Analyses

Arterial hemolymph flow was measured with a pulsed-Doppler flowmeter (545C-4, University of Iowa). This minimum invasive technique was shown to yield reproducible data of hemolymph flow through arteries in decapod crustaceans verified by in situ calibration (Airriess et al. 1994; Reiber et al. 1997). To find optimal positions for Doppler probes and to quantify vessel diameters the anatomy of the arterial system was studied by Batsons no. 17 polymer (Polysciences) casts.

Anterior and lateral arteries are situated directly below the carapace, 2 cm cranial from the heart. Doppler probes (20 MHz, Iowa Doppler Products) for the anterior and one lateral artery were fixed in grooves formed on the outside of the carapace by a moulding cutter without injuring the hypodermis. Invasive preparations were necessary for flow determinations in hepatic and sternal arteries. Small holes were drilled into the carapace directly in front of the heart for one hepatic and into the first abdominal somite for the sternal artery. Holes were covered with latex dam to prevent hemolymph loss. Doppler probes were brought close to the arteries by feeding them through the holes in polyethylene tubing (i.d. 0.6 mm, o.d. 0.9 mm). For a maximum signal piezoelectric crystals of the probes were positioned at a 45° angle to the arteries and fixed with dental periphery wax. The ultrasonic signal was focused by fine tuning to the center of the hemolymph stream for all four arteries

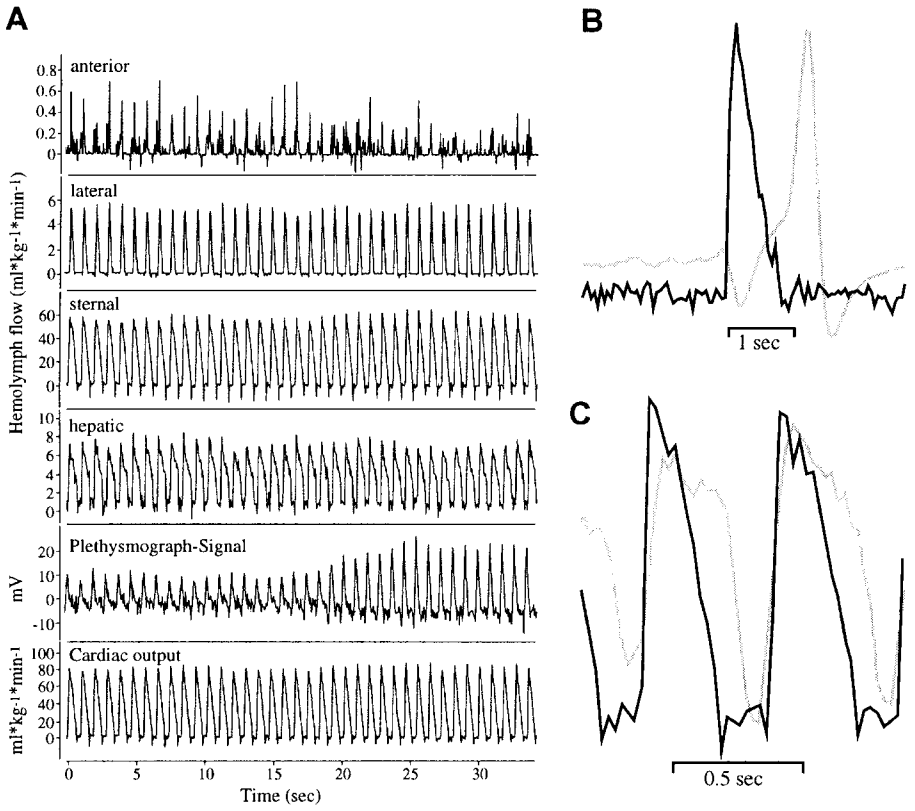

Figure 2. *A*, Comparison of simultaneous recordings of hemolymph flow in four different arteries, photoplethysmograph signals, and calculated cardiac output at 12°C. At constant cardiac output, the plethysmograph signal fluctuates spontaneously. *B*, Cardiac output (*black line*) and plethysmograph signal (*grey line*) for one heart beat at 2°C. At low frequencies, maximum cardiac output coincided with the first negative peak of the plethysmograph recording. *C*, Cardiac output (*black line*) and plethysmograph signal (*grey line*) for two heart beats at 20°C. At high frequencies, the amplitude and pattern of the plethysmograph signal changed but cardiac output still runs ahead of the large positive pulse.

analyzed. Heart rate was evaluated from the phasic Doppler signal and was monitored by the photoplethysmograph technique as described previously (Depledge 1984; Frederich et al. 2000*a*).

Data Analysis and Statistics

The phasic output of the Doppler flowmeter, the photoplethysmograph signal and temperature were recorded simultaneously by a MacLab system (AD Instruments) at a frequency of 40 Hz per channel. Hemolymph flow was calculated from the diameters of the respective arteries (anterior: 1.0 ± 0.1 mm, lateral: 1.2 ± 0.1 mm, sternal: 2.3 ± 0.1 mm, hepatic: 1.1 ± 0.1 mm). The velocity was evaluated by the Doppler equation $(V = [F_d C][2F_o \cos A]^{-1}$, where V is the velocity in mm s^{-1}, F_d is the Doppler shift frequency in kHz [instrument was calibrated to 0.5-V phasic output per kHz of Doppler shift], C is the velocity of sound in blood [1,565,000 mm s^{-1}], F_o is the transmitter frequency [20,000 kHz], and A is the angle between sound beam and velocity vector [45°]). Mean values of the phasic signal were calculated for each artery at temperature intervals of 0.1°C. Cardiac output and stroke volume were calculated by summing the mean values from all arteries normalized to kilograms of body weight. Heart rate was evaluated as the number of peaks of plethysmograph or Doppler signals per minute. Because signal amplitude of the plethysmograph depends on the intensity of heart movements, plethysmograph signal amplitudes were compared with cardiac output calculated from Doppler signals.

Data were tested for the significance of differences by ANOVA or ANCOVA (SuperAnova, Abacus Concepts 1991) at the $P < 0.05$ level. Results are given as means ± SD. Standard

Table 1: Hemolymph flow in *Maja squinado* under resting conditions at 12°C

	Control Animals ($[Mg^{2+}]_{HL}$ 39.9 ± 4.6 mmol L^{-1})		Mg^{2+}-Reduced Animals ($[Mg^{2+}]_{HL}$ 6.1 ± 0.7 mmol L^{-1})	
	Hemolymph Flow (mL kg^{-1} min^{-1})	Percent of Cardiac Output	Hemolymph Flow (mL kg^{-1} min^{-1})	Percent of Cardiac Output
Anterior artery	.43 ± .19	3.1 ± 1.3	.25 ± .08	.56 ± .2*
Lateral arteries (2)	2.55 ± 1.62	36.8 ± 11.7	1.89 ± .67	8.67 ± 3.1*
Sternal artery	4.04 ± .72	29.1 ± 5.2	23.47 ± 2.97*	53.79 ± 6.8*
Hepatic arteries (2)	2.15 ± .24	31.0 ± 1.7	8.07 ± 2.77*	36.98 ± 12.7
Cardiac output	13.88 ± 2.43	...	43.64 ± 5.01*	...
Heart rate (min^{-1})	73.20 ± 10.7	...	75.80 ± 4.5	...
Stroke volume (mL kg^{-1} min^{-1})	.19 ± .04	...	.58 ± .07*	...

Note. For the calculation of cardiac output flow rates determined in lateral and hepatic arteries are doubled since two vessels exist. Asterisk indicates a significant difference between control and $[Mg^{2+}]_{HL}$-reduced animals (ANOVA, $P < 0.05$).

deviations of cardiac output and stroke volume were calculated by the Gaussian law of error propagation.

Results

Anatomy of the Circulatory System

Six arteries leave the heart of *Maja squinado*, one sternal and one anterior artery as well as two lateral and two hepatic arteries. The sternal artery runs from the heart downward to the ventral side of the animal where it becomes the ventral thoracic artery. About 2 cm below the heart the ventral abdominal artery branches off the sternal artery. This ventral abdominal artery supplies hemolymph to the abdomen while the ventral thoracic artery leads hemolymph through different branches into the walking legs. Further cranial the large ventral thoracic artery bifurcates in two branches circumventing the esophagus and supplying hemolymph to some mouth parts, including the scaphognathite. The anterior artery runs cranial directly below the carapace without any ramifications and hemolymph of this vessel is led to the supraesophagial ganglion. For about 2 cm both lateral arteries are running parallel with the anterior vessel. Then they deviate toward the musculature of the stomach and supply hemolymph also to the cephalic appendages. The hepatic arteries originate below the heart and lead hemolymph directly into the hepatopancreas. Inside this organ both arteries are connected before they dissipate into a well-developed ramified artery system. To our knowledge this junction of the two hepatic arteries has not been described before. The posterior artery running from the heart to the abdomen, usually developed in brachyuran crabs, is largely reduced in *Maja* (McLaughlin 1983). Hemolymph flow in this vessel was not confirmed according to polymer casts or nuclear magnetic resonance flow-weighted imaging (M. Frederich, C. Bock, H. O. Pörtner, unpublished observations).

Plethysmograph Signals and Cardiac Output

The amplitude of the photoplethysmograph signal showed a pattern of correlation with cardiac output that was not uniform for all animals investigated (Fig. 1). Signal amplitudes varied between animals because they depend on the position of the sensor in relation to the heart and are also influenced by carapace thickness. In some animals signal amplitude increased with cardiac output, whereas it decreased in others. Simultaneous recordings of cardiac output (Doppler) and plethysmograph signals are shown in Figure 2. The sequence of events indicates that large peaks recorded with the plethysmograph at low heart rates were probably correlated with diastolic filling of the heart while systolic hemolymph ejection coincided with the initial small and negative pulse (Fig. 2*B*). At faster heart rates (at higher temperatures) this differentiation between systole and diastole disappeared (Fig. 2*C*). Maximum cardiac output signals still ran ahead of plethysmograph maxima, especially when considering that the Doppler recordings occurred downstream of the heart and were delayed compared with heart contraction.

Heart Rate

Mean heart rates under resting conditions at 12°C were the same in control and $[Mg^{2+}]_{HL}$-reduced animals (Table 1). Both groups showed a reduction in heart rate with temperature (Fig. 3), however, with some significant differences. Heart rate decreased significantly in control animals (ANOVA, $P < 0.05$) between 12° and 7°C with a major drop at 8°C followed by a plateau when no further decrement occurred down to 4.5°C (Fig. 3). At lower temperatures the significant (ANOVA, $P < 0.05$) decrease in heart rate continued. A further significant (ANOVA, $P < 0.05$) plateau period appeared between 2° and 3°C when heart rate was maintained at 10.9 ± 1.5 beats per

minute. The increase in heart rate seen below 1°C was not accompanied by a rise in hemolymph flow (see below). The disproportionate decrease at 8°C and the subsequent plateau were not found in $[Mg^{2+}]_{HL}$-reduced animals. Heart rate fell progressively and significantly in this group, however, with a major drop only between 6° and 4°C. In the figure the downward shift of this break point temperature is indicated as a shift in pejus temperature T_p (see "Discussion"). Heart rates were only slightly but nonsignificantly higher in $[Mg^{2+}]_{HL}$-reduced animals between 12° and 6°C (ANCOVA, $P < 0.05$). Between 5° and 3°C they were slightly but nonsignificantly lower than in control animals.

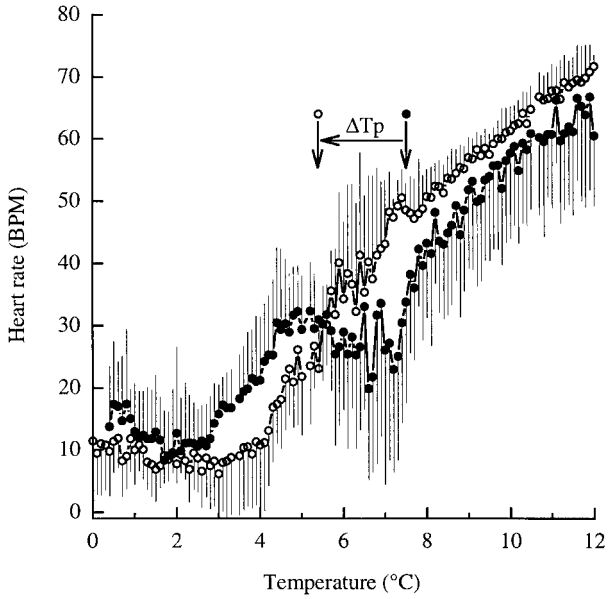

Figure 3. Heart rates of *Maja squinado* at different $[Mg^{2+}]_{HL}$ during progressive cooling at 1°C h^{-1} (*filled circles*, controls at 39.9 ± 4.6 mmol L^{-1}, $n = 7$; *open circles*, $[Mg^{2+}]_{HL}$-reduced animals at 6.1 ± 0.7 mmol L^{-1}, $n = 7$). The elevation of heart rate in $[Mg^{2+}]_{HL}$-reduced animals between 6° and 12°C and vice versa between 5° and 3°C was nonsignificant (ANCOVA, $P > 0.05$). The sudden decrease of heart rate in controls at 8°C is followed by a plateau at constant heart rate. In $[Mg^{2+}]_{HL}$-reduced animals, this plateau was not observed, and a disproportionate drop in heart rate occurred below 6°C. An arrow indicates the shift of the break point, which is concluded to represent a downward shift in pejus temperature (ΔT_p, see "Discussion").

Hemolymph Flow

Mean arterial hemolymph flow under resting conditions at 12°C is given in Table 1. The change in hemolymph flow during cooling varied between arteries and groups of animals. In control animals flow fell gradually in the anterior artery (Fig. 4*A*). In lateral arteries flow remained constant between 12° and 8°C and reached a minimum value after a significant decrease between 8° and 7°C. Similarly, a major drop occurred at 8°C in sternal and hepatic arteries after a more continuous decrease between 11° and 8°C. In $[Mg^{2+}]_{HL}$-reduced crabs this range was shifted to between 6° and 5°C when flow through the anterior and hepatic arteries decreased drastically (Fig. 4*B*). A more gradual decrease in flow occurred in lateral and sternal arteries. Hemolymph flow through sternal and hepatic arteries remained higher in $[Mg^{2+}]_{HL}$-reduced compared with control crabs at all temperatures (see below).

Furthermore, the distribution of hemolymph between arteries was different in the two groups. In control animals, the sternal artery and the hepatic and lateral arteries each received about 30% of the cardiac output at temperatures between 12° and 8°C (Fig. 5*A*; Table 1). At 8°C, flow shifted from lateral arteries to sternal and hepatic vessels. At temperatures below 3°C, the fraction of cardiac output directed to the hepatopancreas increased even further.

In $[Mg^{2+}]_{HL}$-reduced animals, cardiac output was elevated (see below), leading to significantly (ANOVA, $P < 0.05$) higher (four- to 10-fold) flow rates in sternal and hepatic arteries at 12°C, whereas flow through lateral and anterior arteries remained unchanged compared with controls (Fig. 4*A*, 4*B*). As a consequence, both lateral arteries received approximately 10% of the cardiac output at all temperatures except between 5° and 4°C when an increase in the variability of flow through lateral and sternal arteries caused this value to rise to about 20%. Between 5° and 2°C, the fraction directed to the sternal artery rose drastically and was about 40%–50% of cardiac output, slightly higher than the value found in control crabs. Again, the change in flow distribution shifted from 8° to 6°C with $[Mg^{2+}]_{HL}$ reduction.

Cardiac output as the sum of hemolymph flow through all six arteries leaving the heart fell during cooling as shown in Figure 6*A*. Compared with controls, cardiac output in $[Mg^{2+}]_{HL}$-reduced crabs was fourfold higher, between 10° and 12°C, and a major reduction appeared at 6°C rather than at 8°C, again depicted as a downward shift of pejus temperature T_p (see "Discussion"). Stroke volume was significantly (ANCOVA, $P < 0.05$) elevated at all temperatures in $[Mg^{2+}]_{HL}$-reduced crabs (Fig. 6*B*). A transient significant reduction of stroke volume occurred between 7° and 3°C in control animals, while no significant change could be detected in $[Mg^{2+}]_{HL}$-reduced crabs.

Discussion

Methodology

Cardiac output is the most important measure of cardiac performance. The noninvasive technique of photoplethysmography has been used in various studies to monitor cardiac activity in crabs (Depledge 1984; Depledge and Andersen 1990; Aagaard et al. 1991; Aagaard 1996). Infrared light penetrates the carapace

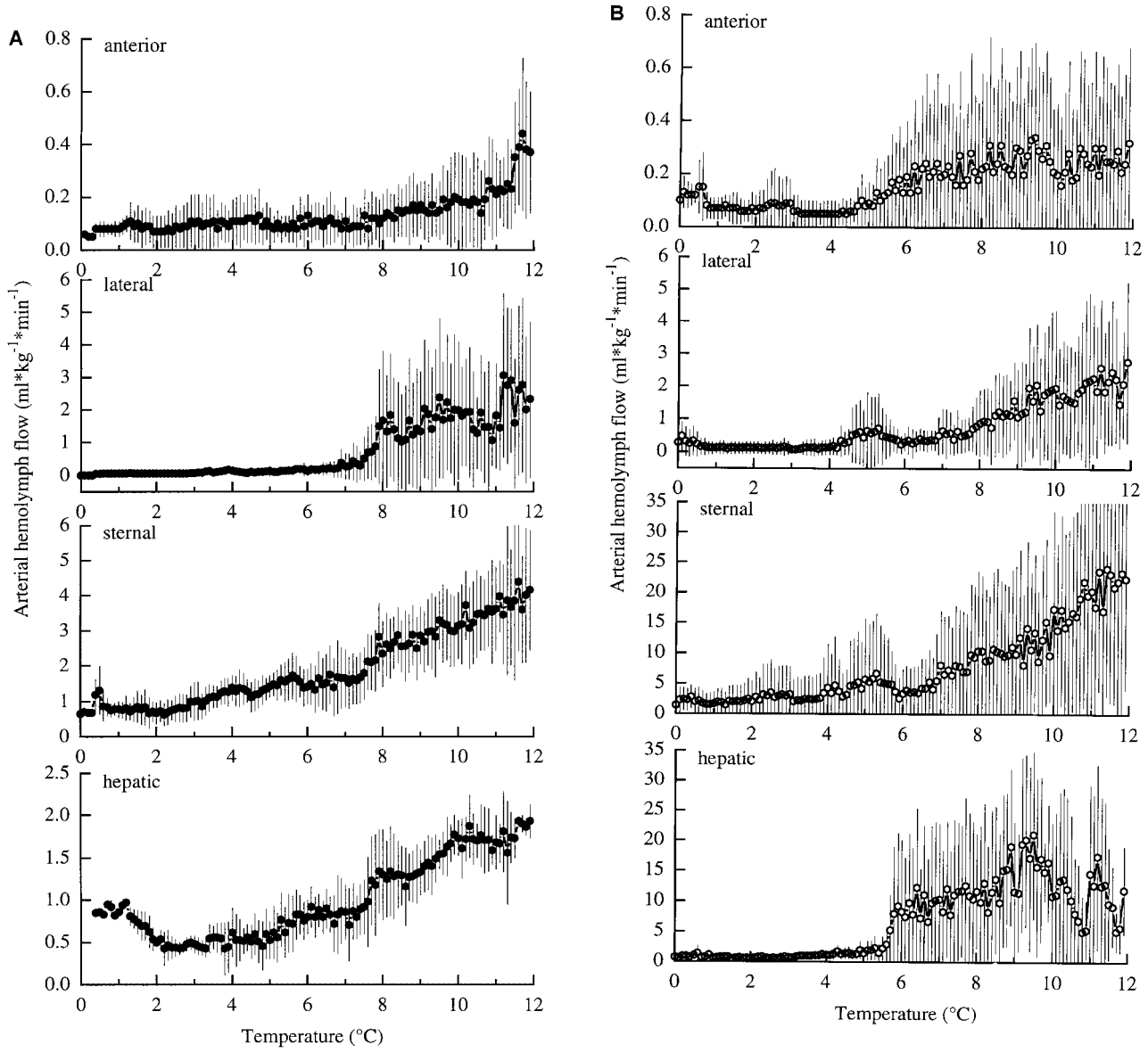

Figure 4. *A*, Hemolymph flow in four different arteries of *Maja squinado* during progressive cooling (control animals, $n = 7$). A major decrease was visible at 8°C in lateral, sternal, and hepatic vessels. *B*, Hemolymph flow in four different arteries of *Maja squinado* during progressive cooling ($[Mg^{2+}]_{HL}$-reduced animals, $n = 7$). A major decrease occurred at 6°C, especially in anterior and hepatic vessels (note the different scales for sternal and hepatic arteries compared with *A*).

and is reflected by the heart. The plethysmograph signal is mainly caused by changes in the shape of the heart during diastolic filling, while systole is detectable only at low heart rates and elicits rather small deflections of the recording (Fig. 2*B*). However, changes in signal amplitude vary largely between specimens, are not consistently correlated with cardiac output, and are, hence, not suitable to monitor this parameter. Therefore, we used the pulsed-Doppler technique to estimate cardiac output from measurements of hemolymph flow during progressive cooling.

The rather rapid and progressive temperature change used in this study does not represent a situation experienced by the animals in their natural environment. Animals had no time to acclimate to lower temperatures as they do during seasonal temperature fluctuations. Therefore, biochemical adaptations like those reviewed by Prosser (1991) or seasonal changes in

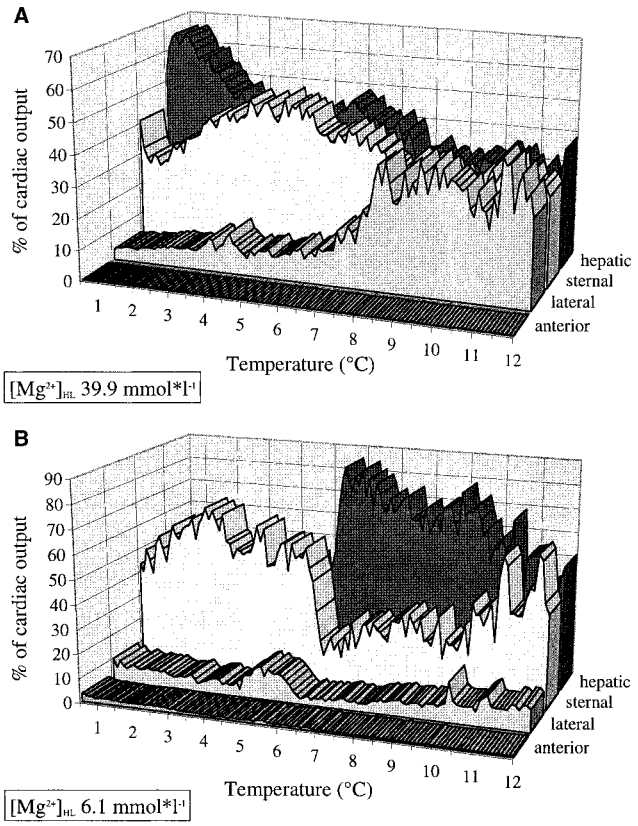

Figure 5. Distribution of hemolymph flow between arteries during progressive cooling (see text). *A*, Control animals, $[Mg^{2+}]_{HL}$ 39.9 ± 4.6 mmol L^{-1}. *B*, $[Mg^{2+}]_{HL}$-reduced animals, 6.1 ± 0.7 mmol L^{-1}.

cardiac thermal sensitivity could probably be minimized. Break points in cardiac performance and thermal limits, which will shift depending on those adaptational processes, should also remain largely unaffected by the experimental protocol.

Heart Rate and Cardiac Output

To our knowledge these are the first data on cardiac output and stroke volume reported for *Maja squinado*. The values are similar to those found in the somewhat bigger Dungeness crab *Cancer magister,* which showed cardiac outputs between 8.6 and 14 mL kg^{-1} min^{-1} and stroke volumes between 0.12 and 0.22 mL kg^{-1} min^{-1} at 12°C (Airriess and McMahon 1994, 1996; Airriess et al. 1994; McGaw and McMahon 1995; DeWachter and McMahon 1996*a*, 1996*b*; DeWachter and Wilkens 1996). Higher values were reported for the crayfish *Procambarus clarkii* by Reiber et al. (1997; cardiac output: 252 mL kg^{-1} min^{-1}, stroke volume: 1.98 mL kg^{-1} min^{-1}) and for the larger *Homarus americanus* (cardiac output: 93.6 mL kg^{-1} min^{-1}, stroke volume: 0.7 mL kg^{-1} min^{-1}; Reiber et al. 1997; Reiber and McMahon 1998).

Cardiac performance decreased progressively during cooling until a break point was reached at 8°C with a disproportionate reduction in heart rate and cardiac output and a redistribution of hemolymph flow in control animals. This break point (called "pejus temperature" [T_p] by Frederich and Pörtner 2000) coincides with the onset of a fall in hemolymph Po_2 and indicates transition from optimum to a progressively deleterious range ("pejus range"; Frederich and Pörtner 2000) associated with a reduction in aerobic scope for activity. Further cooling revealed a plateau between 7° and 4.5°C with constant heart rate and hemolymph flow in sternal and hepatic arteries. This may indicate temperature compensation and lead to constant hemolymph Po_2 (Frederich and Pörtner 2000). It remains open for debate whether this active process enables *M. squinado* to survive colder temperatures for longer periods. Instead of a plateau DeWachter and McMahon (1996*b*) found a strong increase in heart rate variability at similar temperatures in *C. magister*, which also indicates that the system may start to counteract the decrease in temperature. The regulatory mechanisms remain to be investigated. The redirection of hemolymph flow

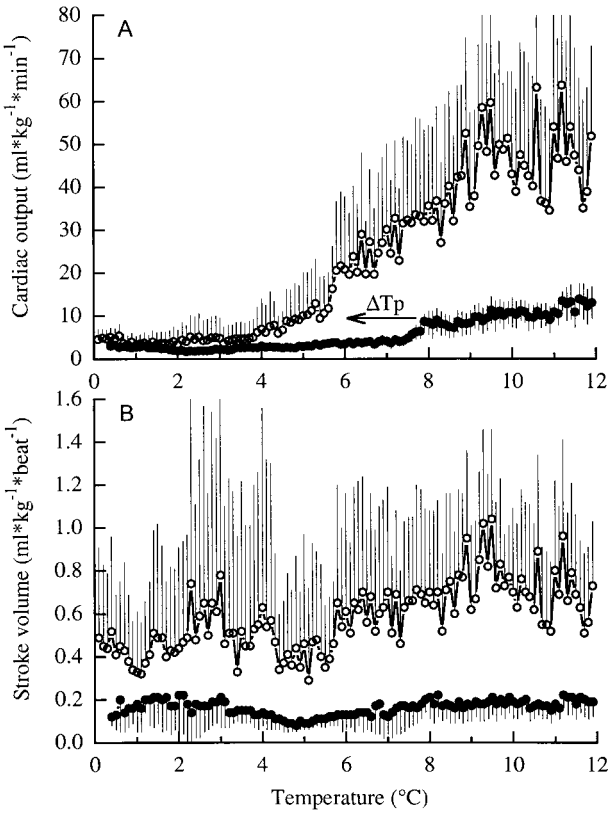

Figure 6. Cardiac output (*A*) and stroke volume (*B*) during progressive cooling in control (*filled circles*) and $[Mg^{2+}]_{HL}$-reduced animals (*open circles*). The downward shift of pejus temperature is indicated by an arrow (ΔT_p, see "Discussion").

from lateral to sternal and hepatic arteries below 8°C indicates an increased perfusion of the walking legs and scaphognathite musculature at the expense of hemolymph supply to the stomach and the cephalic appendages. In the cold, priority may be given to the maintenance of ventilatory and locomotory function. The increased perfusion of the hepatopancreas by the hepatic arteries should prevent early hypoxia in this homeostatic organ. It remains open whether this pattern would be the same after long-term acclimation to low temperatures.

At lower temperatures the animals still survived, but cardiac activity was reduced, especially below 3°C when heart rate reached minimum levels. Finally, transition to anaerobic metabolism occurred indicating that critical tolerance limits were reached (see "Introduction"; Frederich and Pörtner 2000).

Enhancing Cold Tolerance by Reducing $[Mg^{2+}]_{HL}$

The period of metabolic homeostasis indicated by the plateau of constant heart rate and Po_2 in control animals disappeared upon $[Mg^{2+}]_{HL}$ reduction. Moreover, the break point characterized by major reductions of heart rate and cardiac output and by the redirection of hemolymph flow fell from 8° to 6°C. At reduced $[Mg^{2+}]_{HL}$ and 6°C, *M. squinado* displayed the same heart rates as at 8°C under control conditions; however, cardiac output and stroke volume were maintained above control values (Fig. 6). The rise in cardiac performance most likely supported maintenance of hemolymph Po_2. This pattern represents a downward shift of the transition from optimum to pejus (progressively deleterious) temperature range (T_p in Figs. 3, 6). These observations agree well with the correlation between low $[Mg^{2+}]_{HL}$ and high activity levels found in different invertebrates, including crustaceans (Robertson 1953, 1960; Walters and Uglow 1981; Spicer et al. 1994; Frederich et al. 2000*a*). Previously, this correlation was supported by measurements of heart rates and whole-animal oxygen consumption (Frederich et al. 2000*a*). This study corroborates these findings by revealing an increase in cardiac output and stroke volume during $[Mg^{2+}]_{HL}$ reduction. As a corollary, a significant rise in cardiac performance at lower $[Mg^{2+}]_{HL}$ enables brachyuran decapods to be more active at cold temperatures. This allows maintenance of full aerobic scope for activity at lower temperatures, thereby enhancing cold tolerance. In the same way $[Mg^{2+}]_{HL}$ reduction might also cause the low critical temperature to drop when aerobic scope for activity reaches zero and onset of an anaerobic mitochondrial metabolism indicates insufficient oxygen supply (reviewed by Pörtner et al. 1998, 2000). Under control conditions the low critical temperature was reached just below 3°C (Frederich and Pörtner 2000). Maintenance of elevated cardiac output suggests that the critical temperature fell to even lower values at low $[Mg^{2+}]_{HL}$. This shift cannot be quantified by this data; however, the downward shift of the optimum range suggests that $[Mg^{2+}]_{HL}$ reduction in *M. squinado* extended the range of thermal tolerance by about 2°C to lower temperatures.

Low $[Mg^{2+}]_{HL}$ not only caused a rise in cardiac output and stroke volume but also a redistribution of flow between arteries. Elevated flow through hepatic and sternal arteries (Fig. 4B), especially at high temperatures, should support a higher level of metabolism and locomotor activity during $[Mg^{2+}]_{HL}$ reduction. Progressive hypoxia during cooling caused a drop in hepatic flow to control levels and elevated flow to the sternal artery (Fig. 5*B*) in accordance with elevated hemolymph supply to the locomotory system in the cold (see above).

Overall, the changes in hemolymph distribution may be a direct effect of lower $[Mg^{2+}]_{HL}$ because Mg^{2+} affects signal transmission between nerve and muscles (Wernig 1972; Dudel et al. 1982). In decapod crustaceans, arterial valves equipped with innervated muscles prevent the backflow of hemolymph and regulate hemolymph distribution (Alexandrowicz 1932; Kuramoto et al. 1992; Wilkens 1997; Wilkens et al. 1997). The heart is suspended in the pericardial sinus by means of ligaments associated with alary muscles (Alexandrowicz 1932; Maynard 1960). Cardiac output and filling pressure are dependent on tension developed by these alary muscles (Wilkens 1987; Nakamura et al. 1994). Furthermore, pacemaker potentials in the cardiac ganglion neurons of crustaceans are Ca^{2+} dependent (Cooke 1988) and Mg^{2+} is known as "nature's physiological calcium blocker" (Iseri and French 1984). Therefore, it is very likely that changes in $[Mg^{2+}]_{HL}$ exert a direct effect on cardiac performance and the activity of vascular muscles.

Conclusions

Maintaining cardiac performance at a sufficiently high level seems necessary for *M. squinado* to cope with cooling. Cardiac performance is optimum until temperature falls below 8°C. Below this threshold temperature, a disproportionate drop in heart rate and cardiac output cause onset of a decline in hemolymph Po_2 (Frederich and Pörtner 2000). This indicates a decrease in scope for aerobic activity, reflecting transition to a progressively deleterious situation. In this pejus range the animal is still able to survive albeit at largely reduced scope for aerobic activity. The respective threshold temperature ("pejus" temperature, Frederich and Pörtner 2000) agrees well with the low annual mean temperature of 9.1°C in the environment of the investigated population (Dauvin et al. 1991; Sournia and Birrien 1995).

In general, experimental reduction of $[Mg^{2+}]_{HL}$ improves circulatory performance and enables crustaceans to maintain higher levels of cardiac performance at colder temperatures, thereby enhancing cold tolerance. However, hyporegulation of $[Mg^{2+}]_{HL}$ in vivo likely requires significant metabolic energy. In contrast to caridean shrimp and amphipods, this strategy is rarely developed by brachyuran crabs, which lead an energy-saving mode of life. It is discussed elsewhere that these differences in $[Mg^{2+}]_{HL}$ regulation may influence biogeographical distribution patterns, in that it excludes the Brachyurans from

life at subzero temperatures in polar areas (Frederich et al. 2000*a*, 2000*b*).

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